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Abstracts

11th Meeting of the Irish Society of Human Genetics, Friday 12th September 2008

Institute of Molecular Medicine, St. James's Hospital, Dublin.

PROGRAMME:

10.00 – 11.00	Registration / Tea and Coffee
11.00 – 11.05	Welcome
11.05 – 12.00	Spoken Presentations: Plenary I
12.00 – 13.00	**Keynote address:** "Genes for Blood Pressure"
	Professor Mark Caulfield, William Harvey Research Institute, London
13.00 – 14.00	Lunch and Poster viewing
14.00 – 15.30	Spoken Presentations: Plenary II
15.30 – 16.00	Tea and coffee / Poster viewing
16.00 – 16.15	Business Meeting
16.15 – 17.15	**Keynote address**: "Amyotrophic Lateral Sclerosis: Expanded Phenotypes and Complex Genetics"
	Professor Orla Hardiman, National Centre for Neuroscience, Beaumont Hospital, Dublin
17.15 – 18.00	Wine reception / Presentation of Prizes / Meeting Close

SPOKEN PAPERS:

S1. BIALLELIC DELETIONS OF CHROMOSOME 13Q ARE FREQUENT AT DIAGNOSIS IN CHRONIC LYMPHOCYTIC LEUKAEMIA.

Paula Carty, Johanna Kelly, Sarah McCabe, Natasha Coen, Claire Bermingham, Thomas Morris, David Betts

National Centre of Medical Genetics, Our Lady's Children's Hospital, Dublin, Ireland

Chronic lymphocytic leukaemia (CLL) represents the most common leukaemia in the western world. The cytogenetic analysis for specific karyotypic events plays an important part in the diagnostic work up of these patients. The most frequently described aberration in CLL, in about 50% of cases, are deletions of chromosome 13q. Since 2005, 313 patients at initial diagnosis have been investigated at the NCMG using a FISH panel that identifies the common CLL-associated aberrations. One or more aberrations were identified in 238 (77.6%) patients with a deletion of 13q present in 185 (59.1%). Further analysis of the del(13q) patient subgroup showed a remarkable 53 (16.9%) with a biallelic 13q deletion population of cells. In 23/53 patients a cell population with a monoallelic deletion of 13q was also evident. The group of patients with a biallelic deletion differed from the patients with solely a monoallelic deletion in having a notably lower incidence for the presence of other aberrations [2/53 (3.8%) vs. 23/134 (17.2%)]. This latter result indicates that the occurrence of a biallelic 13q deletion arises independently of other recognized aberrations. Given the high incidence of this aberration future studies are needed to assess whether this event has an associated prognostic significance.

S2. APPLICATION OF ARRAY-CGH FOR THE DETECTION OF SUBMICROSCOPIC CHROMOSOMAL IMBALANCES IN 400 CASES OF CHILDREN WITH IDIOPATHIC MENTAL RETARDATION AND CONGENITAL MALFORMATIONS.

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The advent of microarray technology has revolutionised molecular cytogenetics in recent times, accelerating the identification of cryptic chromosomal rearrangements. Enhanced resolution has also led to the identification of many novel new microdeletion and duplication syndromes. Detection rates for chromosome abnormalities with array-CGH in constitutional cytogenetics range from 5 - 20% in individuals with normal results from prior routine cytogenetics testing. We present BAC array-CGH data from approximately 400 cases with unexplained MR following karyotyping and subtelomere MLPA screening. We discuss the identification of novel submicroscopic rearrangements, low-level mosaicism, cryptic translocations, transmission of imbalances (1-4Mb) with no apparent phenotypic effect and the targeting of novel new microdeletion & reciprocal duplication syndromes. Large-scale array-CGH screening has also significantly increased our knowledge on the impact of copy number variants (CNVs), which represents one of the main challenges to differentiate between CNVs that are likely to be pathogenic, and CNVs that are less likely to contribute to an affected individual's clinical presentation. With continual resolution changes, array-CGH will facilitate the identification of novel loci involved in MR and / or malformation syndromes and will provide important insights into the flexibility and plasticity of the human genome.

S3. STUDY OF THE KNOWLEDGE OF INHERITED METABOLIC DISORDERS AMONG PATIENTS AND THEIR FAMILIES IN THE IRISH POPULATION.

Rosie O'Shea[1], Eileen Treacy[2], Anne Marie Murphy[2], Sally Ann Lynch[3], Deborah Lambert[2,3].

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Galactosaemia and Maple Syrup Urine Disease (MSUD) are recessively inherited conditions detected by newborn screening in Ireland. Patients are treated at one centre and genetic information is provided by the specialist team. We assessed knowledge among parents and patients to see whether referral for formal genetic counselling would be beneficial, using a questionnaire including 4 demographic, 8 knowledge, 2 information and 5 disease impact questions. 27 families with galactosaemia and 10 with MSUD were interviewed in clinic. All parents of children with galactosaemia and MSUD answered >75% of questions correctly, but there were misunderstandings about the risk or implications of carrier status. There was a significant difference in knowledge between ethnicities. Adult patients with galactosaemia had more misunderstandings in relation to inheritance, recurrence risks and carrier status than their parents. 83% of study participants requested more information about their condition and its transmission. 40% of affected adults with galactosaemia identified a need to meet others with the same condition. While parents of children with MSUD or galactosaemia are well informed, the majority expressed a wish to be referred for genetic counselling. Adult patients with galactosaemia and parents from an Irish Travelling background could especially benefit.

S4. SHOULD A FAMILY HAVE TO FACE A UV DILEMMA?

Claire W Kirk, Patrick J Morrison.

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With the advancement of high-throughput technologies for mutation screening, unclassified variants (UV; non-informative mutations for which the pathogenic effect is unclear) are increasingly being discovered in the BRCA1 and BRCA2 genes. There are a number of ways to try to establish the pathogenicity of a UV, including testing to see if the UV co-segregates with the disease in a family, checking a number of control samples to exclude its presence in the general population, and accessing websites designed for protein modelling and in silico functional analysis.

In those cases where pathogenicity cannot be established with certainty, there are issues around counselling and management of patients and their families. Genetic centres differ in their reporting policies, with some centres reporting all UVs to patients and others operating on a case-by-case basis. There does seem to be a general consensus that family history remains central to risk assessment and management of family members and that presymptomatic testing using UVs of uncertain clinical significance should not be offered. Here we present a case where a BRCA1 UV was found in a family member affected with breast cancer and discuss our decision regarding disclosure of the result and future management of the family.

S5. A CLOSER LOOK AT MISSINGNESS: THE IMPLICATIONS OF NON-RANDOM MISSINGNESS ON FALSE POSITIVE ASSOCIATION IN GENOTYPE CALLING APPROACHES FOR GENOME WIDE ASSOCIATION DATA.

Richard Anney, Elaine Kenny, Colm O'Dushlaine, Jessica Su, Barbara Franke, Ben Neale, Steven Faraone, Michael Gill.

Department of Psychiatry, Neuropsychiatric Genetics Research Group, Institute of Molecular Medicine & Trinity College Dublin, Ireland.

The considerable data-handling requirements for genome wide association studies (GWAS) prohibit individual calling of genotypes and create a reliance on sophisticated "genotype-calling algorithms". Despite their obvious utility, the current genotyping platforms and calling-algorithms used are not without their limitations. Specifically, some genotypes are not called due to the ambiguity of the data. Any bias in the missing data could create spurious results. Using data from the Perlegen 600K Array - Genetic Analysis Information Network (GAIN) data - we observed that missing genotypes are not randomly distributed throughout the homozygous and heterozygous groups. Using simulation, we examined whether the level and type of missingness observed might influence deviation from the null-hypothesis under common case-control and family-based statistical approaches. Under a case-control model, where missingness is present in a case group but not the controls, we observed bias giving rise to genome-wide significant type-I error for missingness as low as 3%. The family-based association simulations show close to nominal type-I error at 4% genotype missingness. These findings have important implications to study design, quality-control procedures and reporting of findings in GWAS.

S6. GENOME-WIDE ASSOCIATION STUDY USED POOLED DNA SAMPLES AND ITS APPLICATION IN CORONARY HEART DISEASE.

W Meng[1], A Hughes[1], CC Patterson[1], C Belton[1], F Kee[1], PP McKeown[1,2].

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[2] Regional Medical Cardiology Centre, Royal Victoria Hospital, Grosvenor Road, Belfast, BT12 6BA, Northern Ireland, UK.

Background: Genome-wide association studies have been successful in identifying susceptibility loci for common diseases. DNA pooling is a practical way to reduce the huge cost of large-scale genotyping.

Methods: In the first stage, our work focused on the use of 330k / 550k SNP chips, using pooled DNA samples from up to 50 cases or controls. The cases were male patients with early-onset (<55yr) coronary heart disease. The control individuals (age > 68yr) were identified from the PRIME study. We then selected SNPs with differences of allele frequencies between 5% and 13%, combined with a 'cluster' method. In the second stage, replication was undertaken using independent DNA samples from a family-based study (1494 individuals from 580 families with probands having early-onset coronary heart disease).

Results: Among 9 regions, SNPs in 2 chromosomal regions were successfully replicated in the second stage. The P values for SNPs in the DNAJC6 gene, rs501691, rs1325607, rs4325172 were 0.03, 0.03, and 0.004, respectively. Another SNP on chromosome 15, rs3825877 was also positive (P=0.009).

Conclusion: Genome-wide association studies using pooled DNA samples are feasible and may be a cost-effective way to detect genes for complex diseases. DNAJC6 gene may be associated with early-onset coronary heart disease.

S7. POLYMORPHISMS IN THE OXYTOCIN RECEPTOR GENE AND AUTISM: ASSOCIATION AND FUNCTIONAL STUDIES.

Katherine Tansey [1], Matthew Hill, Richard Anney, Michael Gill & Louise Gallagher.

Department of Psychiatry, Neuropsychiatric Genetics Research Group, Institute of Molecular Medicine & Trinity College Dublin, Ireland.

The neuropeptide oxytocin has recently been implicated in the aetiology of autism. We examined 20 markers in oxytocin receptor gene (OXTR) for association in 179 simplex families from the Irish Autism Study. We followed up genetic association studies with allelic expression imbalance (AEI) testing for alterations in expression of the OXTR gene. Using lymphoblast cell lines from the CEU HapMap collection, we examined the influence of common variation and different levels of β-Estradiol and Progesterone on AEI.

We found associations between 3 SNPs in OXTR and autism (rs11720238 p=0.031; rs7632287 p=0.0076; rs4564970 p=0.0091). Two SNPs showed association with a high functioning subset of individuals (rs11720238 p-corrected= 0.025; rs7632287 p-corrected=0.0042). We observed AEI in OXTR. The variation in AEI was driven, in part, by a SNP in intron 3 of OXTR (rs237897; p=0.0265). rs237897 was not associated with autism in our sample. The addition of hormones did not appear to alter AEI significantly from the baseline.

These results confirm the importance of OXTR in the aetiology of autism and identify a SNP involved in differential gene expression.

S8. MODELLING ABERRANT SPLICING IN MUTANT GENES.

CE Willoughby, D O'Prey, DAC Simpson.

Centre for Vision Science, Queen's University Belfast, Belfast, United Kingdom.

Purpose: Up to 50% of all point mutations responsible for genetic diseases cause aberrant splicing. The aim of this study was to model the pathological impact of known mutations on the splicing process in genes associated with Bardet-Biedl syndrome (BBS: MIM 209900). Methods: Computational methods were applied 'in-silico' to prioritise and direct subsequent laboratory workup of mutations in BBS genes to establish which, if any, might have a pathogenic effect on splicing. Splice site score was calculated on 190 exons in 12 BBS genes using web-based servers and correlated with EST evidence. Computational predictions of aberrant splicing were validated in vitro using a minigene system. Results: 290 mutations were modelled and 21% identified 'in-silico' as potential mis-splicing mutations. BBS9, BBS10 and BBS5 genes contained the greatest percentage of possible splicing mutations with 75%, 33% and 33% respectively. A number of predicted aberrant splicing events were modelled with a minigene system in HEK293 cell lines to validate predictions. Conclusions: Traditionally, mutation screening is based on genomic DNA analysis and the effect of a mutation on the mRNA or protein is usually predicted from the primary genomic sequence, as opposed to direct experimental evaluation by determining mRNA expression and splicing patterns. Here, we present an approach to predict and model aberrant splicing in mutant genes.

S9. FUNCTIONAL ANALYSIS OF POLYMORPHISMS IN GENES IMPLICATED IN PSYCHIATRIC DISORDERS.

Matthew Hill, Richard Anney, Michael Gill.

Department of Psychiatry, Neuropsychiatric Genetics Research Group, Institute of Molecular Medicine & Trinity College Dublin, Ireland.

Introduction: Psychiatric diseases such as autism, ADHD and schizophrenia are highly heritable. Past years have seen the identification of a handful of susceptibility genes via traditional candidate gene testing. With the advent genome-wide associations novel susceptibility genes are being rapidly identified. In order to elucidate the molecular mechanisms giving rise to disease susceptibility it is necessary to identify and characterise functional genetic variation. To this end we sought to identify cis acting variation in 'traditional' candidate genes and novel susceptibility genes using allelic expression imbalance (AEI).

Methods: HapMap CEU lymphoblast cell lines were used as a source of mRNA for measuring AEI for the nine selected genes; three monoaminergic genes, a recent schizophrenia susceptibility gene and five novel ADHD susceptibility genes were tested. AEI was determined using TaqMan SNP

genotyping assays.

Results: Significant AEI was observed for 7/9 genes. Only COMT and TRUB1 did not show AEI. A SNP in CHI3L1, rs4950928, previously associated with schizophrenia was strongly associated with AEI.

Conclusion: We have identified cis acting regulatory events in multiple psychiatric disease susceptibility genes. These data will aid in both the future refinement of the association signal and elucidation of the molecular mechanisms underlying susceptibility.

S10. DEVELOPMENT OF GENE THERAPIES FOR DOMINANT DYSTROPHIC EPIDERMOLYSIS BULLOSA.

CP Morgan, D Allen, PF Kenna, P Humphries, GJ Farrar.

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Epidermolysis Bullosa comprises a group of rare and heritable human blistering skin diseases, affecting up to 1 in 17,000 live births. The dominant dystrophic form of EB (dDEB) is characterised by mutations of the COL7A1 gene, resulting in production of mutant type VII collagen protein. Any potential dDEB therapeutic would require suppression of expression of the mutant gene. Continuing advances in the field of gene therapy have led to the discovery of potential therapeutics for dominantly inherited human disorders: the method of RNA interference (RNAi) represents one such molecular tool for suppression of COL7A1 expression. RNAi is based on the sequence specific binding of synthetic RNA molecules to endogenous mRNA transcripts, causing their subsequent degradation. This study involved such suppression in human epidermal keratinocytes, using microRNA vectors expressing short hairpin RNAs targeting COL7A1. Subsequent evaluation of COL7A1 mRNA levels by real time rtPCR showed significant COL7A1 suppression. Additionally, the expression levels of three interferon stimulated genes were evaluated in cells transfected with the COL7A1-targeting constructs. It was found that delivery of these potential therapeutics does not result in an interferon type-1 response. The results obtained thus far represent an important step in the progression towards a suitable therapy for dDEB.

POSTER PRESENTATIONS:

P1. INVESTIGATING PROMOTER HYPERMETHYLATION OF APOPTOTIC GENES IN PROSTATE CANCER.

TM Murphy[1], AS Perry[1] L O'Connor[1], M Lawler.[1]

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It is now well established that cancer cells exhibit a number of genetic defects in the machinery that governs programmed cell death and that sabotage of apoptosis is one of the principal factors aiding in the evolution of the carcinogenic phenotype. A number of studies have implicated aberrant DNA methylation as a key survival mechanism in cancer, whereby promoter hypermethylation silences genes essential for many processes including apoptosis. To date, studies on the methylation profile of apoptotic genes have largely focused on cancers of the breast, colon and stomach, with only limited data available on prostate cancer. The aim of this study was to profile methylation of apoptotic-related genes in order to generate a prostate cancer "apoptotic methylation signature". This in turn could play a role in the early detection and prognosis of prostate cancer and may help elucidate novel therapeutic targets. An in silico approach was first applied to generate a list of apoptotic genes. Relevant genes were identified based on the following criteria: 1) biological role in apoptosis, 2) the presence of a 5' CpG Island 3) susceptibility to promoter hypermethylation in other cancer types and 4) down-regulation in prostate cancer. Under these criteria, 22 apoptotic-related genes were identified as possible targets of methylation in prostate cancer. PCR assays were designed to amplify whole CpG islands in these gene promoters. Genes will be screened for CpG methylation in a panel of prostate cancer cell lines (LNCaP, DU145, PC-3, 22RV1, RC58) using an automated Denaturing High Performance Liquid Chromatography (DHPLC) instrument (WAVE®, Transgenomic Inc). To date, DHPLC results suggest that the CpG promoter region of TMS1, C-FLIP and BNIP3 are fully or partially methylated in the five cell lines examined, while APAF1, CASP8 and CASP3 show no evidence of CpG promoter methylation. Currently we are screening BIK, DR4 and DR5 for promoter hypermethylation.

Genes of interest will be further validated through bisulfite sequencing and methylation levels quantified using quantitative methylation specific PCR in a prostate cancer biorepository that we have generated in Ireland, representing prostate cancer, normal adjacent prostate and benign prostatic hyperplasia.

P2. ANALYZING ILLUMINA HUMAN 1M SNP DATA FOR CNVS USING RAW BEAD-LEVEL DATA.

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With the advent of high-throughput genotyping chips, whole genome association studies are undertaken to determine potential candidate loci by linking common SNPs and copy number variations to human disorders like autism. Illumina's Human 1M SNP beadchip is one of the high-throughput genotyping chips popularly used. The genotype reproducibility for Human 1M SNP chips is 99.99%. However, standard existing methods for prediction of CNVs from the normalized intensity data, generated by Illumina's proprietary software BeadStudio, have between 15-60% reproducible on experimental replicates depending on the algorithm used. We are developing improvements in the pre-processing and normalization of raw intensity bead-level data with respect to improving the CNV reproducibility of experimental replicates. In addition, we are optimizing the normalization of the SNP chip data to recover CNVs predicted using other experimental platforms. Applications to large clinical datasets of probands and parents as well as to HapMap datasets will be presented.

P3. A FUNCTIONAL PROMOTER POLYMORPHISM WITHIN MTHFD1 MAY INCREASE NEURAL TUBE DEFECT RISK IN THE IRISH POPULATION THROUGH AN INTERACTION WITH THE R653Q POLYMORPHISM.

Nicola Carroll[1], Faith Pangilinan[2], Anne M. Molloy[3], James Troendle[4], James L. Mills[4], Peadar N. Kirke[5], Lawrence C. Brody[2], John M. Scott[6], Anne Parle-McDermott[1].

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Genetic variants in MTHFD1 (5, 10-methylenetetrahydrofolate dehydrogenase/ 5, 10-methenyltetrahydrofolate cyclohydrolase/ 10-formyltetrahydrofolate synthetase), a key folate metabolic enzyme, are associated with a number of pregnancy complications, including neural tube defects (NTDs). We have previously reported that a common polymorphism (dbSNP ID: rs1076991 C→T), present in the core promoter region of the MTHFD1 gene, has a negative effect on gene transcription in vitro (ISHG meeting, 2006). We have since investigated this SNP as a potential risk factor for NTDs and report here that it is not an independent NTD risk factor in the Irish population, nor does it influence red cell folate or homocysteine levels. However, SNP-SNP interaction analysis with the previously identified disease-associated SNP rs2236225 G→A (R653Q polymorphism) in the MTHFD1 gene revealed a highly significant association with NTD risk in both case and maternal groups ($P < 0.001$ and 0.01, respectively). These two SNPs are not in linkage disequilibrium and, therefore, the identified interaction cannot be attributed to simple co-segregation. Thus, although not an independent risk factor for NTDs, this SNP is relevant to elucidating the genetic component of common diseases through its interaction with the disease-associated R653Q polymorphism.

P4. ABSTRACT: FUNCTIONAL ASSESSMENT OF S100B AS A SUSCEPTIBILITY GENE FOR PSYCHOTIC BIPOLAR DISORDER.

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Introduction: The glial cell-derived neurotrophic factor, S100B, is implicated in the pathology of bipolar affective disorder (BPAD) and schizophrenia. S100B protein levels are elevated in serum of patients with both disorders

and S100B variants are associated with schizophrenia. We previously reported association of a SNP in the promoter of S100B, rs3788266, with a psychotic form of BPAD (P=0.088). The disease-associated C allele disrupts a Trex/MEF3 consensus recognition, which is bound by Six-family transcription factors, suggesting that it could affect S100B expression.

Methods: The functional effect of rs3788266 on S100B promoter activity was determined using the luciferase reporter system. Promoter fragments containing the T or C alleles of rs3788266 were subcloned into the pGL4.23 minimal promoter-luciferase vector and were assayed for activity in U373MG glioblastoma cells. We also measured S100B RNA levels in post-mortem brain tissue and protein levels in serum to test for possible genotypic effects in vivo.

Results: Luciferase reporter gene expression was significantly increased in the presence of the T compared to C allele (t= 4.151, P=0.001). However, preliminary data indicate that BPAD individuals with the TT genotype have lower mean serum S100B levels compared to those with the TC or CC genotypes (ANOVA: F=5.093, P=0.01). A similar pattern was observed at the RNA level but was not significant.

Discussion: The disease-associated C allele is associated with reduced promoter activity in U373MG glial cells and increased protein levels in serum. SNP rs3788266 may represent a functional susceptibility variant that contributes to the increased S100B levels observed in BPAD patients.

P5. FOUR CASES OF MOWAT-WILSON SYNDROME.

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Mowat-Wilson is a recently described multiple congenital anomaly condition with Hirschsprung disease as a hallmark feature. ZEB2 is the causative gene. We report 4 cases of Mowat-Wilson syndrome. Their age ranged from 22 months to 5 years and 2 months. They all presented developmental delay, microcephaly, typical facial features and absent speech. Interestingly, none had Hirschsprung disease and only one had constipation.¾ had epilepsy which was well controlled and all of these also had congenital heart defects with 2 requiring heart surgery. ¾ had brain imaging with no structural abnormalities were noted. 2/4 had ocular findings, 1 astigmatism and the other a strabismus. All had the typical affable personality. Their developmental delay was in the moderate to severe range.

All 4 were found to have de novo mutations in the ZEB2 gene. Three were novel mutations with 2 being frameshift and the other a nonsense mutation. The fourth mutation had previously been described and was also a nonsense mutation.

P6. VARIANTS IN THE NESTIN GENE AND CORONARY HEART DISEASE.

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[2] Regional Medical Cardiology Centre, Royal Victoria Hospital, Grosvenor Road, Belfast, BT12 6BA, Northern Ireland, UK.

Background: There is evidence that the intermediate filament protein, nestin, may play a role in tissue regeneration and nestin expression has been detected in coronary atherosclerotic plaques. However, to date, no population-based studies concerning the role of the nestin gene in coronary heart disease (CHD) have been reported.

Methods: We evaluated 3 SNPs in the nestin gene amongst 1494 individuals in 580 Irish families with at least one member prematurely affected with coronary heart disease. Genotypes were determined by multiplex SNaPshot technology.

Results: Using the TDT/S-TDT test, we found that rs11582300 and rs3748570 were associated with early-onset CHD (P=0.04 and P=0.02).

Conclusion: We found that nestin gene variants were associated with early-onset CHD. These findings emphasise the importance of further research to explore the role of nestin in atherosclerosis.

P7. INVESTIGATION OF THE PUTATIVE FUNCTIONAL EFFECT OF THE 19BP DELETION POLYMORPHISM WITHIN INTRON 1 OF THE DIHYDROFOLATE REDUCTASE (DHFR) GENE.

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DHFR is an important folate metabolising enzyme that catalyses the conversion of dihydrofolate to tetrahydrofolate. Folate genes are considered candidates for association with neural tube defects (NTDs) such as spina bifida due to the preventative effect of periconceptional maternal supplementation with folic acid. Investigation of an intronic 19bp deletion polymorphism within the DHFR gene found a significant protective effect in mothers of NTD cases when present in one (Relative Risk 0.59 (95% CI: 0.39-0.89), p= 0.01) or two copies (Relative Risk 0.52 (95% CI: 0.32-0.86), p= 0.01). Analysis of mRNA levels revealed a small increase in expression (~1.5 fold) associated with the 19bp intronic deletion polymorphism, but this was not significant (Parle-McDermott *et al., Am J Med Genet* 2007;**143(11):**1174-1180).

We sought to further investigate the potential impact of the DHFR 19bp intronic deletion polymorphism on gene expression by employing a recombinant dual luciferase system in HEK293 cells. The results of these experiments showed that the 19bp deletion showed a modest increase in reporter gene expression in agreement with the mRNA data. It is proposed that the sequence of the 19bp deletion harbours an Sp2 binding site that acts as a repressor of transcription. Mobility shift assays are being employed to directly test whether this polymorphism results in loss of an Sp2 binding site.

P8. UNCOMMON PRESENTATION FEATURES OF THE T(8;14)(Q11.2;Q32) TRANSLOCATION IN ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL).

Johanna Kelly[1], Claire Bermingham[1], Paula Carty[1], Aengus O'Marcaigh[2], Helen Enright[3], David Betts[1]

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The t(8;14)(q11.2;q32) translocation represents a rare but non-random event in ALL that results in a rearrangement of IGH on 14q32. Almost 50% of reported cases occur in either Down syndrome (DS) associated or t(9;22)-positive disease. From a series of over 400 cases of ALL analysed at presentation by G-banding and/or FISH two were found to contain a t(8;14)(q11.2;q32) translocation in either balanced or unbalanced form. Case 1 was an 18-year-old DS female who presented with a der(14)t(8;14) as the sole clonal aberration. The second case was a 49-year-old female who presented with t(9;22)-only as the minor stemline clone and a major sideline clone containing a t(8;14) in which the der(8) had a p arm deletion. Subsequent analysis of this case demonstrated only the presence of the t(9;22) stemline clone. This report further reinforces the association of the t(8;14)(q11.2;q32) with both t(9;22) and DS-associated ALL. As the standard der(8) was absent in both cases we speculate whether other cases with this translocation exist but were not fully characterised. The persistence of leukaemia without the rearrangement questions the importance of the event in the disease process. Further cases are needed to understand its potential relevance to the disease process and prognosis.

P9. AN APPROACH TO IDENTIFY NOVEL HUMAN FOLATE RESPONSIVE GENES.

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The molecular details of how low folate status increases the risk of a range of common diseases such as neural tube defects, cardiovascular disease and colorectal cancer remains to be elucidated. In a bid to begin to understand the molecular response of cells to a restricted supply of folate that is physiologically meaningful; an in vitro cell culture model of folate deficiency was employed in combination with gene expression profiling. The nature of the experimental design ensures that the group of genes that initially respond to folate deficiency would be identified.

A Coriell® lymphoblast cell line that was homozygous wildtype for the MTHFR 677C>T polymorphism (CC) was grown in folate sufficient or folate deficient media over a 12 day period. The folate deficient cells were grown in the presence of hypoxanthine and thymidine throughout the 12 day period to ensure the cells maintained a similar proliferation rate to the folate sufficient cells. The hypoxanthine and thymidine were removed several hours prior to harvest on day 12. Each condition was carried out in replicates of 5 yielding

a total of 10 RNA samples for subsequent transcriptome profiling. Each RNA sample was hybridisation to an individual Affymetrix Human Genome U133 Plus 2.0 array. These arrays are one of the most comprehensive whole genome expression arrays; consisting of probe sets that represent over 47,000 transcripts.

The resulting data signals were normalized and sufficient and deficient expression profiles were compared. A total of two gene lists were generated; each subjected to stringent or less-stringent statistical parameters and represent genes that showed a consistent differential gene expression pattern across the replicate samples. The stringent list consists of 4 down-regulated genes and 288 up-regulated genes. The less-stringent list consists of 324 down-regulated genes and 597 up-regulated genes. Based on the experimental design; these genes are likely to represent the initial cellular response to a depleted folate supply. Further bioinformatics analysis and confirmation of expression differences by Quantitative RT-PCR are currently being performed.

In conclusion, our approach has identified a list of novel folate responsive genes under conditions of relatively mild folate deficiency. These genes/pathways are likely to represent the initial response of a cell to low folate status. These results will ultimately lead to a better understanding of how an individual's folate status influences their disease risk.

P10. MOLECULAR ANALYSIS OF KERATOCONUS IN NORTHERN IRELAND.

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Purpose: Keratoconus (KC; MIM#148300) is the commonest reason for corneal transplantation in the Western world. Mutations in the visual system homeobox gene 1 (VSX-1; MIM#605020) and superoxide dismutase 1 (SOD1; MIM#147450) have been reported in KC. The purpose of this study was to perform a comprehensive screening of VSX-1 and SOD1 in KC patients and further molecular analysis of the chr15q22 linked to a Northern Irish family we mapped previously.

Method: Index cases with KC were recruited and mutational analysis of VSX1 and SOD1 gene was carried out. Further candidate gene analysis was performed in chr15q22 interval and the region was analysed for copy number variations (CNV).

Results: Four VSX1 sequence variants c.432C>G (p.D144E), c.479G>A (p.G160D), c.789C>T (p.S263S) were not seen in 100 healthy controls. Segregation was not detected for p.D144E and also for an intronic changes, c.844-13T>A. Although predicted to alter VSX1 splicing p.S263S had no effect on transcript processing. A silent mutation in SOD1 was detected in a familial KC patient and absent from 100 controls. Till date no pathogenic mutations and no CNV detected within the linkage region chr15q22.

Conclusions: VSX1 and SOD1 play a minor role in keratoconus pathogenesis. The identification of the genetic basis of the chr15q22 KC family is ongoing.

P11. VITAMIN D RECEPTOR POLYMORPHISMS FOK 1 AND APA 1 HAVE NO ASSOCIATION WITH SKIN CANCER IN RENAL TRANSPLANT PATIENTS.

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Vitamin D has potent anti-tumour properties. Calcitriol (1,25(OH)2D3), the hormonal derivative of vitamin D3, is an antiproliferative and prodifferentiation factor for several cell types, including human squamous cells of the head and neck. Several polymorphisms of the vitamin D receptor have been described, including Fok 1, Taq 1, Apa 1 and Bsm. These polymorphisms have been reported to be associated with the occurrence and outcome of malignant melanoma[1]. Bsm BB genotype is associated with increased squamous cell carcinoma risk [2]. We have examined the frequency of FokI and ApaI polymorphisms in 401 renal transplant patients and measured the association with squamous cell carcinoma, basal cell carcinoma, melanoma and also renal allograft survival. There was no association between patients with the polymorphism and the development of SCC, BCC or melanoma. There was however significantly improved graft survival at 3, 5 and 10 years for heterozygotes and homozygotes for the T allele (p=0.03) of Fok 1 Vitamin D polymorphism. This is the first time that the Fok I polymorphism has been associated with improved renal allograft survival. The finding is in keeping with a number of other related studies. In a retrospective study patients receiving $1,25(OH)_2D_3$ along with standard immunosuppression had improved graft survival. There is also animal data showing prevention of chronic allograft rejection with use of Vitamin D receptor agonists. Any risk for skin cancer would be compounded for by the longer graft survival and is therefore a true negative association.

1. Osborne JE, Hutchinson PE. Vitamin D and systemic cancer: is this relevant to malignant melanoma? *Br J Dermatol* 2002;**147(2):**197-224.
2. Han J, Colditz GA, Hunter DJ. Polymorphisms in the MTHFR and VDR genes and skin cancer risk. *Carcinogenesis* 2007;**28(2):**390-397

P12. PHENOTYPIC VARIANTS IN MELAS.

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MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes, is one of the commonest mitochondrial disorders. The incidence is estimated to be around 1 in 15,000, but may be higher. The age of presentation and symptomatology vary widely, making under-diagnosis likely. Early-onset stroke is the commonest presentation in adulthood. Presentation in childhood is more unpredictable and non-specific. Early cardiac changes generally begin in childhood but are asymptomatic. We report the case of an individual who was diagnosed following early-onset stroke and epilepsy. The pedigree showed a brother with diabetes mellitus and a sister who died of cardiomyopathy. Testing shows all family members to have a mt3243A>G mutation, the commonest mutation found in MELAS. All have had mutational load measurements in blood and urine. Mutational load may influence prognosis, but does not always correlate with phenotype due to tissue heteroplasmy. This family will be followed up closely, and will have mutational load measured annually. Our patient is currently being treated with anti-epileptic medication, along with aspirin, L-arginine and thiamine. This family illustrates some of the many complexities in dealing with mitochondrial disorders.

P13. A FAMILIAL T(2;9)(Q37.3;Q12) TRANSLOCATION: AN ILLUSTRATION OF THE POTENTIAL LIMITATIONS OF COMMERCIALLY AVAILABLE FISH PROBES.

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The importance of cytogenetic family studies following the identification of either an inherited or de novo unbalanced chromosome abnormality is well documented. Conventional G-band analysis was performed on a 2 day old girl with dysmorphic features, frontal bossing and low set ears. The result indicated the presence of an additional chromosome, identified as del(9)(q12), "trisomy 9p", a recognised syndrome that is typically considered to arise de novo and was consistent with her phenotypic features. G-band chromosome analysis was performed on both parents and showed a maternal reciprocal translocation t(2;9)(q37.3;q12). In an attempt to delineate the translocation further, FISH analysis with subtelomere probes mapping to 2q and 9q (TelVysion, Vysis) was undertaken. Unexpectedly, the 2q subtelomere region had not been translocated to the der(9). Given the breakpoints involved in this case, if only FISH and some molecular based studies were performed, the potential for a familial t(2;9) translocation would have remained unsuspected with significant consequences for this family.

P14. ABNORMALITIES OF 3Q26 IN MYELOID MALIGNANCY: THE BELFAST EXPERIENCE.

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Rearrangements of chromosome 3q26, although well recognised in myeloid disease, are still relatively rare. They have been reported in AML, MDS, CML and other MPDs. They tend to be associated with tri-lineage dysplasia, prior treatment with alkylating agents, shorter survival time, poor response to treatment and abnormal expression of the EVI1 gene. However, in the majority of patients demonstrating abnormal EVI1 expression, 3q26 rearrangements are generally not detected cytogenetically.

Since 1990 the Northern Ireland Regional Cytogenetics lab has investigated 11 patients with 3q26 rearrangements. These patients (6 females and 5 males) suffered from a range of myeloid conditions including AML of various subtypes, MDS and transforming CML. Age of diagnosis ranged from 55 to 86 years. Survival data was available for 7 patients and ranged from <1 to 21 months post diagnosis.

Our experience therefore confirms the poor survival generally associated

with 3q26 rearrangements and enforces the importance of establishing their presence at disease diagnosis and during treatment. Technical difficulties have precluded the development of satisfactory FISH tests in the past, however, new molecular technologies are now available. Considering the poor prognosis, routine introduction of these techniques for cultures that fail to grow or patients with a normal karyotype should therefore be considered.

P15. AN ATTENUATED FORM OF MORQUIO DISEASE SEEN IN NORTHERN IRELAND.

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Morquio disease (mucopolysaccharidosis type IV) is a lysosomal storage disorder causing predominantly skeletal manifestations. It is caused by a deficiency of galactosamine-6-sulphate sulphatase. In the classical form of Morquio disease there is extreme short stature with average height being between 90 and 120 cm. There are marked skeletal deformities and many affected individuals require surgery to stabilise their cervical spine. We have identified 6 individuals in Northern Ireland who have an attenuated form of the disease – two sets of siblings and two single cases. Ages ranged from 27 years to 38 years. Height ranged from 142 cm to 160cm. This form of the disease was initially considered relatively benign. However it is now clear that affected individuals have major problems with their joints and 5/6 patients have had at least one major joint replaced with two having had 3 joints replaced. Our patients have also shown evidence of osteoporosis. We will present biochemical and molecular data on the cases.

These patients were all initially considered to have spondyloepiphyseal dysplasia before the correct diagnosis of MPS IV was made.

P16. MULTIPLEX MASSARRAY SPECTROMETRY (IPLEX) PRODUCES A FAST AND ECONOMICAL TEST FOR THE DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLAEMIA.

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An iPLEX™ assay that includes 57 mutations in the LDLR, APOB and PCSK9 genes, gives a 75% detection rate for definite Familial Hypercholesterolaemia (FH) and was tested to determine if this technology was applicable to routine genetic diagnostics. The iPLEX™ MassARRAY platform (Sequenom GmbH) utilizes single base extension of mass modified terminators using MALDI-TOF mass spectrometry to analyse primer extension products, determining SNPs accurately, rapidly and economically.

The iPLEX™ test was verified by analyzing 150 FH samples with a previously characterized mutation and 96 no-mutation control samples. Mutations were identified in all 150 FH mutation-positive samples using the iPLEX™ assay, with 96% directly called by the software. The false positive rate was 0.015%, and the overall specific mutation assay failure rate was 2.1%.

116 hyperlipidaemia patients with elevated cholesterol levels were tested by the FH iPLEX™ assay, with 21 (18%) having mutations identified. This pick-up rate would be significantly increased were the patients to be selected using the Simon Broome criteria. The FH iPLEX™ system chip can test up to 86 patients in approximately two days at a cost of less than €10 per sample, and so provides a useful and efficient first-line screen for FH.

P17. CHALLENGES IN GENETIC COUNSELLING ARISING FROM THE RISK OF MULTIPLE GENETIC CONDITIONS IN A SINGLE FAMILY.

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During a Genetics consultation ascertaining a family history can be a delicate process. Three families in whom several separate genetic risks were present and the complexity of the counselling required are discussed. Family 1 were referred due to a child born with Trisomy 21. The couple also had a family history of SMA and CF and advanced maternal age was also a consideration. Family 2 were referred due to a history of X-linked Adrenoleukodystrophy. A child had also died neonatally due to SMA. Family 3 were referred due to a diagnosis of Fragile X. Further exploration showed a relative affected with Huntington Disease and a neonatal death which revealed, although was unexplained by, a Robertsonian translocation.

Each of these families came to the Genetics clinic with a single genetic condition as their primary focus. Introducing the concept of additional significant yet diverse risks in a clear and understandable manner presented a number of practical counselling challenges particularly with regard to communicating burden of risk.

P18. NON-MOSAIC TRISOMY 22 IN A LIVE-BORN MALE.

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Non-mosaic trisomy 22 is recognised as a common cause of first trimester miscarriage; survival beyond this is rare.

In this case, a baby boy was delivered at 39 weeks by caesarean section to a 39 year-old mother, and survived approx 60 minutes. She had 2 first trimester miscarriages, a daughter with multiple problems including microcephaly, septo-optic dysplasia and severe epilepsy (46,XX), and a healthy 23 year-old daughter from a previous relationship. Scan at 14 weeks was normal, but rescan at 26 weeks showed IUGR. Further assessment at 27 weeks identified abdominal left isomerism; biventricular AV connection, large VSD, single outlet right ventricle; oligohydramnios and severe IUGR. Amniocentesis was declined.

Birth weight was 1390g. He was dysmorphic, with dolicocephaly, hypertelorism, epicanthic folds and an open mouth. The palate was narrow and high, with thickened gums. He had very low set ears; the right ear was rudimentary and the left dysplastic with a preauricular tag. The anus was posteriorly placed and imperforate. Post-mortem was declined. A blood sample was taken for karyotyping and non-mosaic trisomy 22 confirmed.

Survival to term and significant life expectancy in trisomy 22 is usually associated with mosaicism. Identification of non-mosaic cases, and recognition of the poor prognosis, is important to allow clinicians and parents to plan appropriate management.

P19. DOES NOGGIN CAUSE TWINNING?

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Human monozygotic twins account for 1 in 250 live births and are considered genetically identical. The origin of MZ twins is attributed to two or more daughter cells of a single zygote undergoing independent mitotic divisions, leading to independent development and births. To date, the cause of monozygotic twins remains unknown. Human beings and armadillos are the only mammals where monozygosity occurs.

We have previously reported two unrelated sets of monozygotic twins from spontaneous dizygotic triplet pregnancies, with a clinical phenotype of facio audio symphalagism (FAS). The dizygotic sibling of each triplet set has a normal phenotype. Spontaneuosly conceived triplet pregnancies occur in 1 in 6500 births in the Republic of Ireland. Noggin mutations occur in approximately 1 in 10000 births. The probability of FAS recurring in monozygotic twins from a triplet pregnancy is therefore highly unlikely.

A nonsense mutation in the Noggin (NOG) gene located on chromosome 17 q22 has been implicated as a causative factor in FAS. Mutations in GDF5 on chromosome 20q11.2 have also been identified in this syndrome.

We examine the correlataion between the phenotype of FAS and Noggin and monozygosity to detemine if a Noggin mutaion predisposes to twinning or indeed if there is an underlying mechanism that might cause noggin to influence monozygotic twinning. It is documented that monozygotic twins show DNA methylation disturbance, for example, Beckwith Weideman Syndrome (BWS) show excess of monozygotic twins (often discordant for BWS) more than by chance. Methylation disturbance has been shown to be the causative factor. As there is a CpG island present in the Noggin gene, one possibility would be to explore the methylation status of Noggin to determine if there are methylation irregularities that may play a role in twinning.

The coding region of Noggin was amplified in two overlapping segments. Sequencing was carried out using the ABI Prism BigDye Terminator Sequencing Kit. Both sets of twins returned normal results at the Noggin

DNA sequencing level. GDF5 sequencing is currently ongoing. Monozygosity of twins was confirmed using the Promega PowerPlex 16 kit.

DNA methylation is currently being assesed in both sets of monzygotic twins and, where available, unaffected siblings, by using both methylaytion microarray chip and BiSulphite modification PCR.

We propose to examine the methylation irregularities of Noggin, and to determine if there is a correlation between noggin methylation and twinning.

P20. THE DESIGN OF A THERAPEUTIC STRATEGY FOR DOMINANTLY INHERITED RETINITIS PIGMENTOSA FOR USE IN LARGER ANIMAL MODELS.

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Retinitis Pigmentosa (RP) represents a group of retinal disorders that results in progressive loss of vision due to photoreceptor cell death. Over 100 mutations have been identified in the rhodopsin gene that gives rise to autosomal dominant (ad) RP. One therapeutic approach which has been suggested for adRP, utilises suppression and replacement. Both wild-type and mutant rhodopsin alleles are suppressed and simultaneously a replacement gene, refractory to the suppression agent is delivered. This strategy has previously been employed in our laboratory using RNA interference (RNAi) as the suppression agent. In this study we decided to design a strategy for use in a porcine model of RP. Two siRNA molecules were designed to target both human and porcine rhodopsin. Initially these were tested in vitro in HeLa cells and one of these siRNAs was found to suppress rhodopsin significantly both at the RNA and protein levels. We are currently converting this siRNA into both shRNA and miRNA formats in order to test suppression in vivo in a mouse RP model carrying a mutant porcine rhodopsin transgene.

P21. WITHDRAWN

P22. AAV-MEDIATED CHRONIC OVER-EXPRESSION OF SNAP-25 IN ADULT RAT DORSAL HIPPOCAMPUS INCREASES EXTRACELLULAR GLUTAMATE AND IMPAIRS SPATIAL LEARNING.

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Long-term memory is formed by alterations in glutamate-dependent excitatory synaptic transmission, which is in turn regulated by SNAP-25, a key component of the SNARE complex essential for exocytosis of neurotransmitter-filled synaptic vesicles. Both reduced and excessive SNAP-25 activity has been implicated in various disease states that involve cognitive dysfunctions such as ADHD, schizophrenia and Alzheimer's disease. Here we provide evidence that over-expression of SNAP-25 in the adult rat dorsal hippocampus, achieved by infusion of a recombinant AAV vector, causes selective impairment in spatial memory acquisition in the water maze task. This effect was accompanied by a specific and significant increase in the levels of extracellular glutamate detectable by microdialysis. These results suggest that chronic high expression of SNAP-25 in a significant proportion of the glutamatergic hippocampal neurons creates a high background transmission state that obscures the spatial memory trace and prevents accurate synapse selection during the consolidation phase.

P23. A 5Q DELETION WITH A CRYPTIC ETV6/RUNX1 IN A CASE OF CHILDHOOD ALL.

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A 7year old girl presented with pallor, puffy face and pain in her foot. Haematological findings showed a WCC of $43.8x10^9$/l, platelets $31x10^9$/l, haemoglobin 5.2g/dl, with 87% blasts. Bone marrow revealed an L1 FAB type with flow cytometric results in keeping with precursor B-ALL (common ALL).

Cytogenetic analysis showed del(5q) and del(12p) in 6/20 (30%) cells analysed. Interphase FISH analysis revealed the presence of ETV6/RUNX1 rearrangement in 193/201 (96%) cells, and of these 161 (80%) showed a deletion of the non-translocated chromosome 12 homologue. FISH using an EGR1 probe for 5q31 showed a del(5q) in 96/200 (48%) of cells examined.

The ETV6/RUNX1 rearrangement is a recurrent finding in childhood ALL, and is associated with a favourable prognosis. Deletions of 12p are a common secondary finding in childhood ALL, however, deletions of 5q are more commonly associated with myeloid disease and have only rarely been reported in ALL.

As the percentage of cells containing the t(12;21) (96%) was so much higher than those containing del(5q) (48%) and also slightly higher than those containing del 12p (80%), this might suggest that the del 5q, and possibly the del(12p), represent secondary abnormalities in this patient. Secondary abnormalities are generally associated with disease progression and therefore influence disease prognosis. The significance of this finding in this particular case remains to be determined.

P24. DEVELOPMENT OF A RETINITIS PIGMENTOSA (RP) GENOTYPING MICROARRAY AND DETECTION OF KNOWN AND NOVEL MUTATIONS IN A COHORT OF NORTHERN IRISH RP PATIENTS.

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The inherited retinal disease Retinitis Pigmentosa (RP) is extremely genetically heterogeneous, with 20 genes implicated in non-syndromic recessive RP to date. However, it is very difficult to identify the causative mutation when a new patient presents. Therefore, we have developed an Affymetrix customseq microarray capable of resequencing reported mutations and the exons within which they are found. In total 30 kbp from 22 genes were tiled on the array. Approximately 100 amplicons spanning the regions of interest were amplified from 35 DNA samples from recessive or sporadic RP patients. These were pooled, fragmented, labelled and hybridised to the genechip according to the manufacturer's protocol. Results were analysed using the GeneChip DNA Analysis Software (GDAS, Affymetrix). An average call rate of 91% was achieved for all the sequences analysed. A total of eleven known mutations in RGR, USH2A and CRB1 and a number of novel sequence variants were detected. This microarray platform is therefore a rapid and effective screen for RP mutations and provides a new tool to include in screening strategies. It is anticipated that the improved detection of RP mutations will facilitate genotype : phenotype correlations, better prognosis and application of therapeutic interventions such as gene therapy.

P25. DETECTION OF A 12P13 CHROMOSOME ANOMALY INVOLVING ETV6 AT RELAPSE IN AN ADOLESCENT PRESENTING WITH CYTOGENETICALLY NORMAL, FLT3-ITD$^+$, TYPE A NPM1$^+$ DE NOVO AML (M5 FAB-TYPE).

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Although pre-treatment MRC AML 10 cytogenetic data has been extremely useful for predicting initial response to chemotherapy, remission duration and overall survival in AML, several studies now indicate that cytogenetically normal AML (CN-AML) is in fact represented by underlying molecular mutations. The clinical impact of NPM1$^+$ (favourable) and FLT3 (adverse) mutations in younger de novo CN-AML adults is now well established.

12p13 chromosomal rearrangements associated with treatment related AML have only been reported in 6 cases and are generally associated a poor median survival (~4 months). To our knowledge, all 6 patients had 12p13 rearrangements resulting from translocations in which a 5'ETV6-3'partner fusion gene was generated.

We describe a CN-AML (M5 FAB-type) 16 year old presenting with two FLT3-ITD mutations and an NPM1 (Type A) mutation. She received intensive chemotherapy but relapsed after 10 months. Despite being FLT3-ITD$^-$, NPM1$^+$, a pericentric inversion of one chromosome 12 homologue was detected with breakpoints at 12p13 and 12q13. Metaphase FISH studies confirmed ETV6 gene involvement.

To our knowledge, this is the first reported case of a 12p13 rearrangement resulting from a pericentric inversion of chromosome 12 at relapse appearing with a persistent NPM1 mutation and loss of FLT3-ITD.

P26. GENOME-WIDE ASSOCIATION (GWA) STUDY OF ATTENTION DEFICIT HYPERACTIVITY DISORDER-COMBINED TYPE (ADHD-CT); ADJUSTMENT FOR GENETIC HETEROGENEITY IN LARGE MULTICENTRE STUDIES.

Richard Anney, Matthew Hill, Colm O'Dushlaine, Elaine Kenny, the IMAGE Consortium, Michael Gill.

Department of Psychiatry, Neuropsychiatric Genetics Research Group, Institute of Molecular Medicine & Trinity College Dublin, Ireland.

As modern humans have spread throughout the world, allele frequencies and linkage disequilibrium (LD) have become more varied between populations. Population admixture is a major source of bias in the case-control study design. Family-based designs, such as the Transmission Disequilibrium Test, have been used to limit this phenomenon. However, the power of the TDT to detect disease susceptibility loci (DSL) can be influenced by population admixture through its impact on the degree of LD between the genetic marker and the DSL.

We have examined the population clustering of a large multicentre study of approximately 950 simplex ADHD-CT families collected from a white-European population. We have applied selection criteria to reduce heterogeneity at the clinical and genetic level.

We present data from this pre-cleaning step and discuss the implications to large multicentre studies. Moreover, we present the results of the application of this data to GWA data using data generated from the IMAGE ADHD-CT study. These data are examined at the marker, gene and hypothesis-free and hypothesis-driven gene-network analysis. Moreover, we examine the functional variation of genes tagged by associated SNP markers.

P27. PARTIAL TRISOMY FOR THE 17Q SUBTELOMERE REGION: FIRST CASE REPORT AND REVIEW OF THE LITERATURE.

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Complex and isolated partial trisomy 17q subtelomere patients have rarely been reported in the literature. We report a patient with an isolated partial trisomy of the 17q subtelomere region. The patient first presented for genetics assessment aged 13 months with abnormal head shape, subtle dysmorphism and normal development. Cranial radiology was normal. Reassessment at 8 years and 9 months showed the patient to have a specific learning disability, mild truncal hypotonia, gross and fine motor skills delay, atrial septal defect, trigonocephaly with prominent metopic ridge and dysmorphism. There is a history of similar learning difficulties and motor delay in an older sister. Chromosome analysis and 22q11 and 2q37 FISH deletion studies were normal. Subtelomere testing using multiplex ligation-dependent probe amplification showed a duplication of the 17q subtelomere probe region. Further FISH testing demonstrated that the patient had an unbalanced translocation between chromosomes 13p and 17q resulting in a partial trisomy for the 17q subtelomere region. Parental chromosomes were normal. To our knowledge, this is the first reported patient with an isolated partial trisomy of 17q subtelomere. It also demonstrates the value of reassessment of patients in light of new technology.

P28. NOVEL SPLICE SITE MUTATIONS AS THE CAUSE OF FAP-RELATED CANCER IN TWO FAMILIES.

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Familial Adenomatous Polyposis (FAP) is an autosomal dominant inherited disease which is characterized by the presence of adenomatous polyps in the colon and rectum. Here we describe two novel mutations in the adenomatous polyposis coli (APC) gene in two separate families which have been shown to affect RNA splicing and be the cause of the FAP-related cancer.

In one family, a 62-year old woman diagnosed with Attenuated FAP was referred for testing. DNA sequencing of the APC gene revealed a recently identified mutation c.423G>T (p.R141S) in exon 4. The mutation was subsequently found in the proband's sister, niece and nephew. In the second family, a 51-year old male diagnosed with an adenocarcinoma of the right colon and the presence of multiple polyps (50+) in the whole colon was tested. DNA sequencing of APC gene revealed a c.1409-5A>G (p.G470VfsX15) mutation at the intron/exon boundary of exon 11. Reverse transcriptase-PCR demonstrated at the RNA level that both mutations affected the splice site. One mutation resulted in deletion of exon 4 of APC gene and the other mutation created an alternative splice acceptor site at the 5' end of exon 11.

P29. A ROLE FOR GENETIC VARIATION AT HOMER2 IN SCHIZOPHRENIA: FURTHER EVIDENCE FROM IRISH AND OTHER EUROPEAN POPULATIONS.

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Schizophrenia (SZ) is a complex disorder of uncertain aetiology but which may involve dysfunction at glutamatergic synapses of the brain. Based-on linkage and gene expression data we identified HOMER2 (OMIM 604799) as a plausible candidate gene for SZ. Homer2 is enriched at excitatory synapses where it links glutamate receptors to the cytoskeleton. We previously reported allelic and haplotype evidence of association at HOMER2 in a sample of 375 cases and 812 cases from Ireland (Gilks *et al. Ulster Med J* 2008;**77(1):**66[S7]). The best result was at rs869498 (p=0.016, OR 1.39)

The International Schizophrenia Consortium conducted a genome-wide association study of schizophrenia using 3,380 cases and 3,593 controls from Europe (Affymetrix 5.0 and 6.0 platforms). Across HOMER2, 44 SNPs were genotyped of which 11 were associated with disease status at $p<0.05$. Of our four previous LD-independent associations, two (at rs2306428 and rs869498) were reproduced by proxy (rs17158194, p=0.001, OR 1.15 and rs17158155, p=0.02, OR 1.27 respectively). We have also found evidence for association with genes regulated by HOMER2 in this dataset. These data support a role for HOMER2 in SZ susceptibility and further genetic and functional studies are warranted to investigate molecular pathways involving HOMER2 in SZ.

P30. ALLELIC EXPRESSION IMBALANCE ANALYSIS OF PSYCHOSIS SUSCEPTIBILITY GENES.

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Schizophrenia and bipolar disorder are complex psychiatric disorders affecting approximately 1-2% of the population worldwide. Evidence from twin and family studies indicate that both disorders are highly heritable. However, identifying candidate genes associated with the disorders has proved difficult due their complex genetic aetiology. The advent of genome wide association studies (GWAS) has made is possible to rapidly identifying potential candidate genes associated with common disorders. It now remains to further investigate these regions to elucidate the putative functional roles of susceptibility variants. As part of international consortia for schizophrenia and bipolar disorder research we are investigating several genes (ZNF804A, CACNAC1 and ANK3) that have been significantly associated in GWAS for possible functional variants. Genetic variants that have a regulatory role in gene expression can be investigated by measuring Allelic Expression Imbalance (AEI), whereby the expression levels of two alleles from a marker SNP can be compared in heterozygous individuals. The presence of AEI indicates cis acting factors that influence gene transcription or processing. Heritable differences in gene expression are thought to contribute to the susceptibly of many complex diseases and could potentially influence an individuals susceptibility to schizophrenia.

P31. LARGE SCALE PATHWAY-BASED ANALYSIS OF GENOME-WIDE ASSOCIATION STUDY DATA: IMPLICATIONS AND APPLICATIONS.

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With the advent of whole genome association studies, listing of all results for up to 1 million SNPs is not feasible and usually only the top associated SNPs are listed/ analysed in-depth. Such focused analysis may hide the biology behind disease.

To overcome this problem we have designed a novel SNP ratio test (SRT) to look at all significant SNPs in a biologically relevant format. The SRT analyses the significant SNPs that lie within genes in KEGG pathways, and looks for enrichment of significant to non-significant signal in one pathway compared to all other pathways.

We applied this method to 7 whole genome association studies testing which pathways were significantly enriched for association signal in the different datasets. The association data was sourced from the Wellcome Trust Case Control Consortium that carried out a joint whole genome association study examining 2,000 individuals for each of 7 major diseases using a shared set of 3,000 controls.

It is striking to note that obvious pathway candidates for certain diseases e.g. Type I diabetes mellitus pathway and Type II diabetes mellitus pathway were both found to be significant in the Type I diabetes and Type II diabetes datasets respectively.

P32. NOVEL GENOMIC PATHWAY ANALYSIS OF GENOME-WIDE ASSOCIATION STUDIES: IDENTIFICATION OF ERBB SIGNALLING AS A SUSCEPTIBILITY PATHWAY IN PARKINSON'S DISEASE

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Background: Systems-based approaches to mining complex genome-wide association study (GWAS) data have recently gained prominence. We have developed, and evaluate the performance of a novel SNP ratio test (SRT), which compares observed to expected ratios of significant to non-significant SNPs within versus outside pathways, in two Parkinson's disease (PD) GWAS datasets.

Results: In both PD datasets the SRT identified significant evidence for involvement of the "ErbB signalling pathway" in PD aetiology. Comparison with an alternative method of pathway analysis, genomic pathway mining (GPM), provided convergent statistical support for involvement of this pathway in PD. The erbB signalling pathway performs multiple neuronal functions and has been linked to many neuropsychiatric disorders including Alzheimer's disease, schizophrenia and PD.

Conclusions: We identify the SRT as a valuable method of rapidly identifying pathway signals in GWAS data, which can be followed up using more detailed pathway modelling to identify the relative contributions of different SNPs to susceptibility. This approach may be particularly valuable for complex genetic disorders of uncertain aetiology and is applicable to any available pathway resources. In PD, further replication and characterisation of involvement of erbB signalling pathways is warranted in larger, more densely genotyped datasets.

P33. NO EVIDENCE OF ASSOCIATION BETWEEN POLYMORPHISMS IN A 2P25 GENE-CLUSTER WITH VARIATION IN BONE MINERAL DENSITY

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Osteoporosis is a complex skeletal disease, under strong genetic influence, that alters the mineral composition of bone resulting in a loss of bone mineral density (BMD), variation in bone strength and elasticity which leads to an increase in non-traumatic fractures. We chose to fine map a gene-cluster at 2p25 to elucidate potential genetic associations with BMD variation. The potential candidate genes in this 387.6kb region are the development and differentiation enhancing factor 2 (DDEF2), integrin beta 1 binding protein 1 (ITGB1BP1) and a disintegrin and metalloproteinase domain 17 (ADAM17). Haploview was used to determine the gene-cluster linkage disequilibrium structure and select tagSNPs. The Kbioscience Genotyping service was employed to determine the SNP genotypes in 552 individuals from 251 families (192 pedigrees) ascertained through probands with low BMD (T<-1.5). Sixteen tagSNPs captured 70.0% of the HapMap validated variation across the gene-cluster region. None of the SNPs significantly deviated from HWE. Age, height, weight and sex were included as covariates in all subsequent statistical analysis. There was no evidence of population stratification, linkage or association observed between these tagSNPs or haplotypes and the BMD phenotypes tested. Denser SNP genotyping and replication in an independent study is required to support these results.

P34. EPIGENETIC SUPPRESSION OF CTNNA3 AND ITS NESTED GENE LRRTM3 IN UROTHELIAL CARCINOMA OF THE BLADDER (UCB).

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LRRTM3 is normally neuronally expressed and is located within the largest intron of CTNNA3 suggesting co-evolution of these two genes. LRRTM3 is entirely located within CTNNA3, but is transcribed in the opposite direction and is therefore a nested gene. Moreover, CTNNA3 is a developmentally imprinted gene, with preferential expression of the maternal allele, while LRRTM3 is not imprinted.

Taqman® QRTPCR employing the relative quantity method was used to determine the mRNA levels of CTNNA3 and LRRTM3 in a series of UCB cell lines (HT1376, RT4, T24, TCCSUP, RT112, CAL29). The demethyl transferase inhibitor 5-aza-2'deoxycytidine (DAC) was used for the chromatin modifying treatments of TCCSUP.

We demonstrate that CTNNA3 and LRRTM3 are co-ordinately expressed in these UCB cell lines. In TCCSUP, mRNA levels of CTNNA3 and LRRTM3 are minimal. However, following DAC treatment, CTNNA3 and LRTMM3 demonstrated increased mRNA expression by 4 and 7 fold respectively. Two CpG islands identified in the promoter region of CTNNA3 (MethPrimer) show no evidence of DNA methylation following sodium bisulphite modification and sequencing. Therefore, the increased expression of CTNNA3 following DAC treatment suggests indirect effects of this drug on this region such as the demethylation of transcription factors or transcription factor binding sites common to both genes.

P35. RET VARIATION IN THE AETIOLOGY OF VESICOURETERIC REFLUX.

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Vesicoureteric reflux (VUR) is the retrograde flow of urine from the bladder towards the kidneys and is a major cause of renal failure and hypertension. Primary VUR is a developmental anomaly of the vesicoureteric valves and commonly occurs along with other developmental anomalies of the urinary tract in the same individual or other members of the same family. The cause of VUR is unknown but it often runs in families and may be inherited as an autosomal dominant in most cases. Some of the genes already known to be involved in urinary tract development are also involved in other developmental processes and therefore their mutation is liable to cause multiple anomalies and is unlikely to result in isolated VUR. RET is such a gene. Some mutations of RET result in multiple endocrine neoplasia, and others in Hirschsprung disease (defective intestinal innervation). However, a group in Quebec found that a single nucleotide polymorphism (SNP) in RET, which changes an amino-acid (p.Gly691Ser), is greatly increased in VUR, with a heterozygote frequency of 69% as against 29% in the healthy Quebec population. We present the results of a study of this SNP in VUR patients and

healthy controls in the Irish population and discuss the implications.

P36. THE SEARCH FOR GENES INVOLVED IN VESICOURETERIC REFLUX.

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Vesicoureteric reflux (VUR) is the retrograde flow of urine from the bladder towards the kidneys. It is common in young children and is a major cause of renal failure and hypertension, though the condition resolves in some as they grow. Primary VUR is a developmental anomaly of the vesicoureteric valves and is part of a spectrum of developmental anomalies of the urinary tract. Though a few genes are known whose mutation causes VUR in addition to defects of other organs (such as renal-coloboma syndrome, and branchio-oto-renal syndrome), the cause of isolated VUR is unknown, but genetic studies so far suggest that it is highly genetically heterogeneous. A genome scan that we performed on 129 Irish families highlighted 10-15 regions of the genome that appeared to show linkage to the disorder, including 2 regions yielding non-parametric lod scores >2.5. We investigated the genes and non-coding regulatory elements in these regions to develop a priority list of places in which to search for possible pathogenic mutations, and present the results of our search so far.

P37. PHENOTYPE AND GENOTYPE ANALYSIS OF FAMILIAL PERIODIC PARALYSIS IN IRISH FAMILIES.

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The familial periodic paralyses are caused by autosomal dominant mutations of skeletal muscle ion channels leading to altered membrane excitability. The disorders are characterised by episodes of limb weakness and paralysis lasting minutes to hours. Hyperkalaemic periodic paralysis (Hyper PP) is caused by missense mutations in the skeletal muscle sodium channel gene, SCN4A. Hypokalaemic periodic paralysis (HypoPP) is due to mutations in the calcium channel gene CACNA1S (HypoPP1) and mutations in SCN4A (HypoPP2).

We aimed to identify patients and families with periodic paralysis in an Irish population and characterise them clinically and genetically.

Patients were recruited through a neurology tertiary referral centre. Detailed clinical and family history, physical examination and specialised neurophysiologic examination were performed in each case. Genomic DNA was extracted and screening was performed for known and novel gene mutations.

To date we have identified 4 families with HyperPP and 1 with HypoPP (genetically confirmed elsewhere). All of the HyperPP families have the same SCNA4 Met1592Val mutation, but are phenotypically heterogeneous. One is a four-generation pedigree with an unusually prolonged attack duration. The HypoPP patient has a de novo but previously reported SCNA4 mutation.

P38. IL18 AND IL2 POLYMORPHISM IN PSORIASIS SUSCEPTIBILITY IN THE IRISH POPULATION.

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Psoriasis is an inflammatory disease of the skin and joints with a prevalence of up to 2% in European populations. IL18 is a pro-inflammatory cytokine, which promotes Th1 T-cell development by inducing γ-interferon. Genetic variation in this gene has been implicated in the pathogenesis of several autoimmune conditions, including inflammatory bowel disease, type 1 diabetes, atopic eczema and asthma. There is some evidence that IL18 may play a role in psoriasis. rs6840968 is a single nucleotide polymorphism (SNP) within the IL2/IL21 region of chromosome 4q27, which has recently been associated with Type I Diabetes, Rheumatoid Arthritis, Graves' Disease and Celiac Disease. The functional relevance of the IL2 and IL21 genes in inflammatory and autoimmune conditions is highlighted by the role of these cytokines in T-cell activation and development. We have genotyped IL18 rs187238 (IL18-137) and IL2/IL21 rs6840968 in 231 ethnically uniform Irish patients with psoriasis and 871 Irish controls. Both loci conformed to HWE in both populations. Both loci showed evidence of association with psoriasis in this population (IL18 rs187238, Odds Ratio 0.61 [0.44 – 0.86], $\chi^2 = 8.99$, $P = 0.0027$; IL2/IL21 rs6840978, Odds Ratio 0.67 [0.48 – 0.92], $\chi^2 = 6.41$, $P = 0.01134$, for carrier status of the minor allele in both cases). This latter value for IL2/IL21 rs6840978 is similar to a recent study of psoriasis susceptibility for the same SNP in UK and US populations (OR = 0.77 [0.62 – 0.95] and OR = 0.81 [0.68 – 0.96], respectively), for the same allele and direction, substantiating existing evidence that the IL2/IL21 haplotype block represents a risk factor for the development of psoriasis.

P39. A RETROSPECTIVE AUDIT OF AUTISM REFERRALS TO THE NORTHERN IRELAND REGIONAL GENETICS SERVICE BETWEEN 1988-2008.

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Autistic spectrum disorder (ASD) comprises impairment in the three core domains of social interaction, language development and behaviour. Prevalence has increased fourfold in the past ten years, likely due to heightened awareness and broader diagnostic criteria. Aetiology is primarily unknown with neurological, metabolic and genetic factors responsible for 5-10%.

Due to the significant increase in ASD referrals to the Genetics Service a retrospective audit of ASD referrals over the last 20 years was completed to develop guidelines for referrers. Referral details, genetic investigations completed, presence of a family history and confirmation of ASD diagnosis were some of the features analysed in the referral, in addition to the outcome of the genetic assessment.

Of the cohort, 60% were familial, 15% isolated and 25% associated with congenital abnormality and or learning disability in the referral letter. 32% had genetic investigation prior to referral. After genetic assessment, 47% were familial, 14% genetic, 39% of unknown aetiology.

We recommend that ASD referrals to the Genetics Service should have the following criteria (1) confirmation of the ASD phenotype (2) completion of chromosomal analysis and Fragile X studies (3) positive family history of learning disability / ASD and /or (4) dysmorphic features / other congenital abnormality.

